Voltage-gated lipid ion channels

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ABSTRACT   Lipid membranes can display channel-like ion conduction events even in the absence of proteins. We show here that these events are voltage-gated with a quadratic voltage dependence as expected from electrostatic theory. To this end, we recorded channel traces and open current histograms. We determined the equilibrium constant between closed and open state and the open probability as a function of voltage.

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Introduction

Synthetic lipid bilayers can display channel-like conduction events through pores in the bilayer (lipid ion channels, reviewed in (1)). These channels are most frequent close to the melting transition of the membranes. The conductance properties are comparable to those reported for protein channels both with respect to channel open lifetime (2–4) and to single channel conductance. It has further been shown that lipid channels are sensitive to drugs such as anesthetics (5) and to changes in intensive variables such as temperature, membrane tension, calcium concentration and pH (see (1)). In this letter we demonstrate that lipid channels are also gated by voltage.

The effect of voltage on black lipid membranes was investigated by several authors in the 1970s. It was found that charging of the membrane capacitor generates a force on the membrane that leads to an effective reduction of the membrane thickness and thereby to an increase in capacitance. It has been proposed that electroporation (the generation of voltage-induce pores in bilayers) is a consequence of these forces (6–9). Recently, we proposed that the electrostatic forces associated with an excess charge can also induce melting transitions in membranes (10). Such transitions would influence the elastic properties of the membrane and thereby the likelihood of pore formation in the membrane (5).

The electrostatic force, $\mathcal{F}$, exerted on a planar membrane by a voltage is given by

$$\mathcal{F} = \frac{1}{2}\frac{C_m V_m}{D} \tag{1}$$

where C_m is the membrane capacitance, V_m is the transmembrane voltage and D is the membrane thickness (10). This force reduces the thickness of the membrane (11). The electrical work performed on the membrane by a change in thickness from D_1 to D_2 is

$$\Delta W_{el} = \int_{D_1}^{D_2} \mathcal{F}dD = \frac{1}{2}\epsilon_0 V_m^2 \int_{D_1}^{D_2} \epsilon\frac{A}{D^2}dD \equiv \alpha V_m^2 \tag{2}$$

where A is area, ϵ_0 is the vacuum permittivity, and ϵ is the dielectric constant of the membrane core. The coefficient α is constant for constant temperature and pressure. This work is proportional to the square of the voltage, and one would thus reasonably assume that the free energy of pore formation is related to the square of the voltage and to the elastic constants of
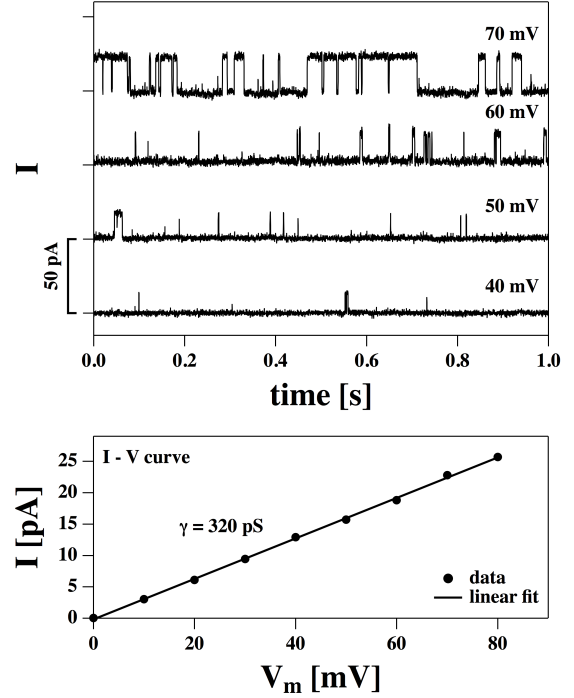

Figure 1: *Top: Current-traces for a DMPC:DLPC=10:1 mol/mol membrane (150mM KCl, T=30°C) at four voltages showing an increase of single-channel conductance with voltage and an increased likelihood of channel formation. Bottom: The corresponding linear single-channel current voltage relation indicating a single-channel conductance of γ=320 pS.*

the membrane. The free energy for pore formation has the form

$$\Delta G = \Delta G_0 + \alpha V_m^2 \tag{3}$$

where ΔG_0 is the free energy difference between open and closed pore in the absence of voltage. ΔG_0 reflects the elastic properties of the membrane that depend on composition, temperature and pressure. Such quadratic dependence is reasonable for symmetric membranes because a linear term can be excluded for symmetry reasons. For asymmetric membranes one rather obtains $\Delta G = \Delta G_0 + \alpha(V_m - E_0)^2$, where E_0 is a reversal potential (12).

The probability, $P_{open}(V_m)$, of finding an open pore in the membrane at a fixed voltage is given by

$$P_{open}(V_m) = \frac{K(V_m)}{1 + K(V_m)} \; ; \; K(V_m) = \exp\left(-\frac{\Delta G}{kT}\right) \; , \tag{4}$$
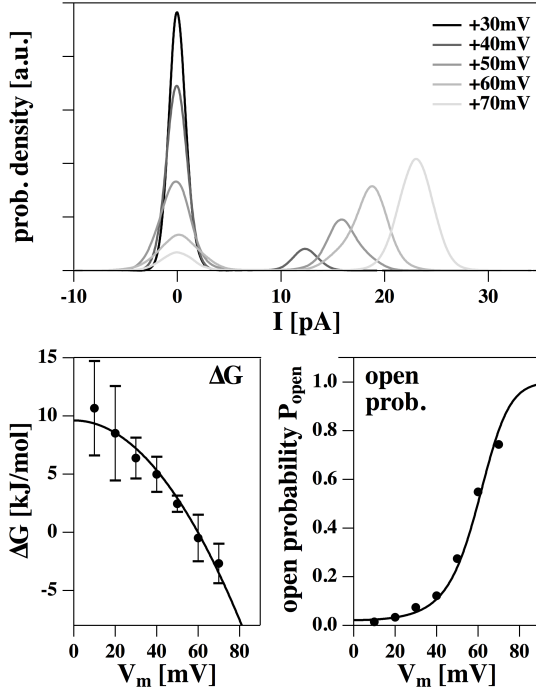
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Figure 2: *Top: Experimental probability distribution for channel opening at five voltages (cf. Fig. 1). Bottom, left: The calculated free energy difference between open and closed state as a function of voltage indicates a quadratic voltage dependence (ΔG_0=9.6 kJ/mol; α=-2.7·10^3 kJ/ mol·V). Bottom, right: The open probality, P_{open}, shows a transition at 60 mV.*

Figure 3: *Recordings on a DMPC:DLPC membrane at $29°C$. Top: Probability distribution for the first (left) and the second open state (right). Bottom: The calculated free energy difference for both conduction steps is consistent with a nearly identical quadratic voltage dependence ($\Delta G_{0,1}$=25.4 kJ/mol; α_1=-22.2·10^3 kJ/ mol·V; $\Delta G_{0,2}$=46.9 kJ/mol; α_2=-18.6·10^3 kJ/ mol·V).*

where $K(V_m)$ is the voltage-dependent equilibrium constant between closed and open states of a pore. Thus, it is expected that the likelihood of channel opening in pure lipid membranes is enhanced by voltage.

In previous reports we have demonstrated that membranes can display non-linear current-voltage relationships (4, 13) and that lipid channels can be induced by voltage. Here, we study the voltage-gating of lipid channels more systematically.

MATERIALS AND METHODS

Lipids were purchased from Avanti Polar Lipids (Alabaster, Al). Electrophysiological measurements were performed on a synthetic membrane spanned over the tip of a patch pipette using the tip-dipping method (14). The patch diameter was ~ 1 μm. The experimental details were described in (4).

RESULTS AND DISCUSSION

We performed patch recordings on a DMPC:DLPC=10:1 membrane (15) at T=30°C in a 150mM KCl buffer. This mixture displays a chain melting transition close to room temperature (4). In Fig. 1 (top) we show current traces of a mem-
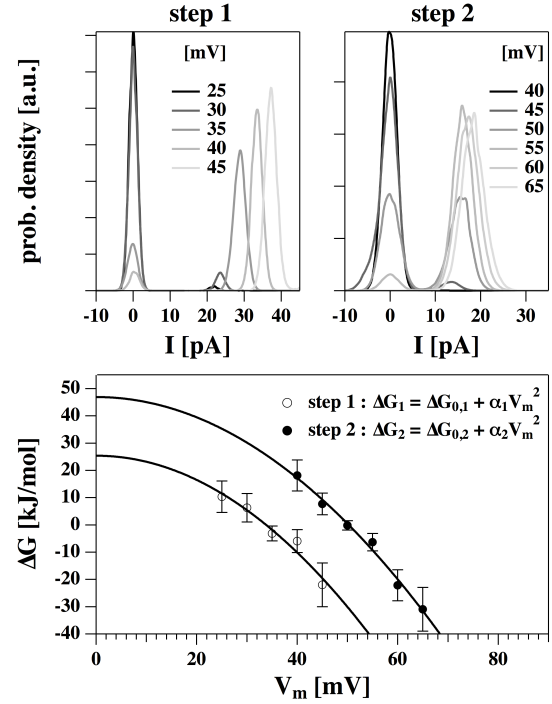
brane recording at four different voltages. The appearance of the recordings is quite stable and can last up to 30 minutes. Increasing the voltage leads to a larger single-channel current and to an increased likelihood of finding open channels. The current-voltage relation for the single-channel current is linear (Fig. 1, bottom), indicating the presence of lipid channels with a constant pore size. The single-channel conductance is γ =320 pS.

Fig. 2 (top) shows the current histograms associated with the current traces of Fig. 1. One finds two peaks in the distribution representing the closed and the open states. The current of the closed state was set to zero. The (voltage-dependent) equilibrium constant ,$K(V_m)$, between open and closed state can be deduced from the peak areas of the two states in the histogram. From the equilibrium constant one can deduce the free energy difference of the two states (Fig. 2, bottom left) and the open probability (Fig. 2 bottom right) — see eq.4. The solid lines in the bottom panels represent fits assuming a quadratic form of the free energy as given by eq 3. Fig. 2 indicates that one finds a voltage-induced transition from a closed to an open state over a range of about 20 mV. Such a range is also typical for the activation of voltage-gated Na^+- and K^+-channels (e.g., (16)).

Fig. 3 shows the current histograms for a different membrane (DMPC:DLPC=10:1; T=29°C) that displayed two conduction steps at different voltages. The top left panel shows the

histograms of the first step (zero pA corresponds to the base-line current). The closed-to-open transition takes place at ∼34 mV. The top right panel shows the current histogram of the second conduction step (zero pA corresponds to the current of the first open channel). This transition takes place at ∼50 mV. The free energy of the two steps is given in Fig. 3 (bottom). The free energy of step 2 represents that of the single channel (i.e., it is the difference in free energy between the first and second open event). Both curves are well approximated by quadratic functions. At zero voltage, the free energy of the second pore ($\Delta G_{0,2}$) has been found to be approximately twice the free energy of one pore ($\Delta G_{0,1}$ — parameters are given in the figure legend). This is also reflected in the fact that the second pore forms at a higher voltage. The parameter α describing the voltage dependence of each pore is the same within error.

SUMMARY AND CONCLUSIONS

We have shown that lipid ion channels in model membranes are voltage-gated. This is in agreement with previous reports on the effect of electrostriction on membrane thinning (6, 10, 11). The free energy of the open pore has a quadratic dependence on voltage. The conductances of single channel events and the closed-to-open transition width are of a similar order to the results found for protein channels. This result complements earlier findings that lipid channels can be gated by other intensive variables, e.g., anesthetic drugs, temperature, pH, and calcium (1).

The quantitative similarities between lipid and protein channels are intriguing. We find it likely that these similarities are not accidental but rather suggest that conduction events have a common origin in the thermodynamics of the biomembrane.

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